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RESEARCH BRIEF

Plasmodium falciparum: Exported Protein-1, a Blood Stage Antigen, Is Expressed in Liver Stage Parasites

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After inoculation by *Anopheles* sp. mosquitoes, *Plasmodium falciparum* sporozoites rapidly make their way to the liver (Fairley 1947) where a single, uninucleate sporozoite develops during a minimum of 5-6 days to a mature liver stage schizont with $1-3 \times 10^4$ uninucleate merozoites. There are no clinical or pathological manifestations associated with this stage of the parasite's life cycle, and thus the parasite developing within the liver is an attractive target for vaccine-induced protective immune responses. Infected hepatocytes are the target of protective immune responses induced by immunization with irradiated sporozoites, sporozoites that develop only partially in hepatocytes (Hoffman *et al.* 1989). However, so-called "erythrocytic stage" parasite proteins such as the *P. falciparum* major merozoite protein-1 (PfMSP-1) are also expressed in infected hepatocytes (Szarfman *et al.* 1988a,b). As such, they also could be the targets of cellular or humoral immune responses that prevent the release of infectious merozoites from the liver. The current studies were undertaken to determine whether the erythrocytic stage *P. falciparum* exported protein 1 (Exp-1) is expressed in infected hepatocytes.

Exp-1 (Simmons *et al.* 1987), also called circumsporozoite related antigen (Coppel *et al.* 1985), QF116 antigen (Kara *et al.* 1988), or antigen 5.1 (Hope *et al.* 1984), is a 23-kDa *P. falciparum* blood stage protein that is secreted by the parasite into the host cell. It accumulates at the parasitophorous vacuole membrane and within vesicles in the infected red cell cytoplasm (Simmons *et al.* 1987). It contains a sequence of 15 amino acids with homology to the tandemly re-

peated tetramers of the *P. falciparum* circumsporozoite protein (PfCSP) (Hope *et al.* 1985). The monoclonal antibody (mAb), 5.1, that recognizes this epitope in Exp-1 also reacts with sporozoites (Hope *et al.* 1985) and prevents the invasion of sporozoites into human hepatocytes (Mellouk *et al.* 1990), presumably by binding to CSP on the sporozoite surface. This cross-reaction has made it difficult to determine whether Exp-1 is itself expressed in infected hepatocytes. We raised antisera against a recombinant Exp-1 which does not contain the epitope cross-reactive with the PfCSP and demonstrate expression of Exp-1 in infected hepatocytes.

The complete coding sequence of Exp-1 was amplified from total RNA isolated by the guanidinium method (Chirgwin *et al.* 1979) of the 3D7 clone of *P. falciparum* strain NF54 using the oligonucleotide primers 5'-GGAATTCATGAAAATCTTATCAGTA-3' and 5'-GGAATTCCTTAGTGTTTCAGGGCCACT-3', cloned into pUC 18 and sequenced (Sanger *et al.* 1977). The sequence is similar to previously published Exp-1 sequences and includes the change of Asp 136 to Gly which abolishes the 5.1 epitope (Simmons *et al.* 1987). The amplified Exp-1 sequence was cloned into the bacterial expression vector, pGEMEX (Promega), and expressed in *Escherichia coli* strain HMS174 (DE3) (Studier *et al.* 1990). In this system, recombinant protein is expressed as an insoluble fusion protein with a 260 amino acid hydrophobic leader from phage T7 gene 10. Recombinant Exp-1 (rExp-1) migrated at its expected size, 50 kDa, in 10% SDS-PAGE and, in immunoblotting, was recognized by the anti-Exp-1 mAb, mAbN1 (Gunther *et al.* 1991) (Fig. 1A).

The rExp-1 protein was electroeluted from a polyacrylamide gel. In addition, the T7 hydrophobic leader protein without the Exp-1 insert was run on a polyacrylamide gel, and this protein and *E. coli* proteins that migrated at the same size as rExp-1 were electro-

Sequence data from this article have been deposited with the GenBank database under Accession No. L15631.

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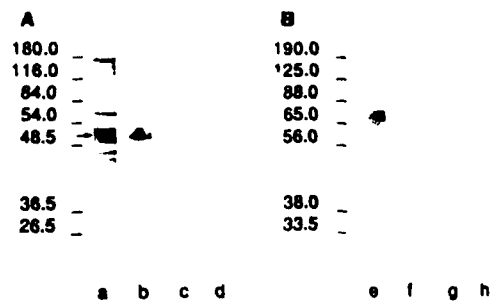


FIG. 1. Western blot of electroeluted rExp-1 (A) and recombinant PfCSP (B) electrophoresed on 10% acrylamide SDS gels. The nitrocellulose strips were probed with (a) mAbN1, (c) NFS1 mAb against PfCSP, (b, f) mouse antisera against rExp-1, (c, g) sera of one of the immunized volunteers before immunization, and (d, h) sera of the same individual post immunization with irradiated sporozoites.

eluted. Anti-rExp-1 and control sera were obtained by immunizing BALB/c mice ip with 50- μ g doses of either rExp-1 or a mixture of the T7 hydrophobic leader protein and the control *E. coli* proteins that ran at the same molecular weight as rExp-1. Proteins were emulsified in complete Freund's adjuvant (first dose) and in

incomplete Freund's adjuvant (second and third doses).

Immunofluorescence assay (IFA) of *P. falciparum*-infected erythrocytes and fixed sporozoites was carried out as described (Szarfman *et al.* 1988a). For IFA of *in vitro*-infected hepatocytes, primary human hepatocytes were obtained in accordance with institutional review guidelines, processed, and infected with *P. falciparum* sporozoites as described (Rogers *et al.* 1992). Cryosections prepared from a *P. falciparum* (clone HB3)-infected chimpanzee liver biopsy that had been kept at -70°C since being obtained by Szarfman and colleagues (Szarfman *et al.* 1988a) were used in an IFA to evaluate the *in vivo* expression of Exp-1 antigen. The IFA was carried out as previously described (Mellouk *et al.* 1990).

Control sera did not react with infected erythrocytes or hepatocytes by immunofluorescence or with extracts of infected erythrocytes by immunoblot (data not shown). The anti-rExp-1 serum did not react with recombinant PfCSP, falc 2.3 (Bathurst *et al.* 1992) by immunoblot (Fig. 1B, lane f), confirming that this serum does not cross-react with the PfCSP. In immunofluorescence, Exp-1 was not detected in sporozoites or in *in vitro*-infected hepatocytes at 2 days postinfection. However, Exp-1 was found in *in vitro*-infected hepatocytes at 4 and 6 days postinfection (results not

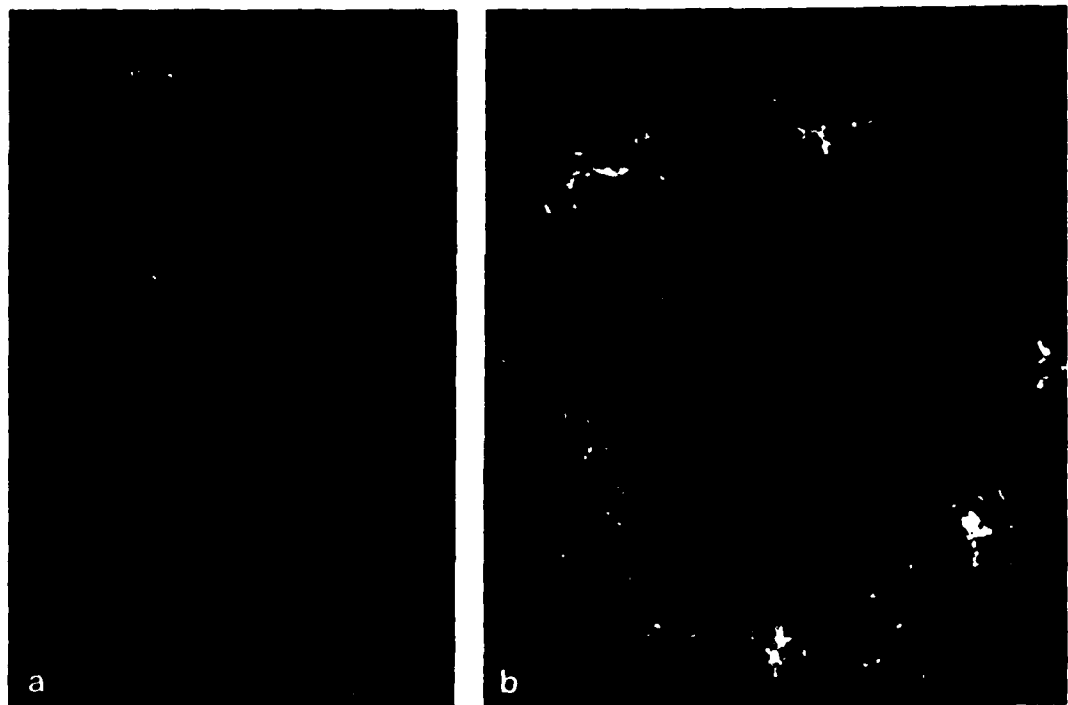


FIG. 2. Immunofluorescence assay on (a) 3D7 *P. falciparum*-infected red blood cells and (b) chimpanzee liver biopsy 6 days postinfection with HB3 *P. falciparum* sporozoites. The slides were incubated with anti-rExp-1 serum and goat anti-mouse Ig fluorescein-conjugated second antibody.

TABLE I
Expression of Exp-1 Antigen in Preerythrocytic and Erythrocytic Stages of *P. falciparum*

	Sporozoite	Hepatic stage (days) ^a			Blood stage
		2 ^b	4 ^b	6 ^{b,c}	
MAbN1	-	-	+	+	+
Mouse anti-rPfExp-1	-	-	+	+	+
Mouse anti-rPfSSP2	+	+	-	-	-

Note. Mice were immunized with rPfExp-1 or rPfSSP2. Recognition of sporozoites, liver stage parasites, and infected erythrocytes in immunofluorescence assays are contrasted.

^a Days after infection with *P. falciparum* sporozoites.

^b *In vitro* *P. falciparum*-infected human hepatocytes.

^c *In vivo* chimpanzee liver biopsy, 6 days postinfection with *P. falciparum* sporozoites.

shown), in red blood cells infected with *P. falciparum* clone 3D7 (Fig. 2a), and in a 6-days-postinfection chimpanzee liver biopsy (Fig. 2b). We have used the same system to express recombinant *P. falciparum* sporozoite surface protein 2 (PfSSP2). Antibodies to rPfSSP2 recognize sporozoites and 2-day *in vitro*-infected hepatocytes, but do not recognize late liver schizonts or infected erythrocytes (Rogers *et al.* 1992) (Table 1). Sporozoite expression of PfSSP2 mRNA was demonstrated by reverse-transcriptase PCR (RT-PCR) and of the protein by Western blot (Rogers *et al.* 1992). The differential recognition of PfSSP2 and PfExp-1 on IFA (Table 1) and the lack of recognition of infected hepatocytes by sera induced by immunization with control proteins strongly support our contention that PfExp-1 is expressed in late liver stages of *P. falciparum*. These findings were substantiated by studies with mAbN1. This mAb reacts in Western blot with a major band of the recombinant Exp-1 protein, but does not recognize any protein in *E. coli* extracts. In IFA assays this monoclonal antibody reacts on infected hepatocytes, as does the anti-rPfExp-1 sera (Table 1). These results show that PfExp-1, originally characterized as a blood stage antigen, is also expressed in hepatic stages.

Since irradiated sporozoites undergo partial development in hepatocytes (Mellouk *et al.* 1991), we wondered whether individuals immunized with irradiated sporozoites develop antibodies against Exp-1. We therefore immunoblotted rExp-1 or the recombinant PfCSP, falc 2.3, with sera from three volunteers immunized with irradiated *P. falciparum* sporozoites (Egan *et al.* 1993). Although the sera strongly reacted with recombinant PfCSP (Fig. 1B, lanes g and h), none of the sera recognized rExp-1 (Fig. 1A, lanes c and d), suggesting that irradiated sporozoites do not develop sufficiently in the hepatocytes to express Exp-1.

Exp-1 has been of considerable interest as a target for malaria vaccine development. Phase I human trials designed to induce protective antibodies against Exp-1

expressed in blood stage parasites have already been conducted (Stürchler *et al.* 1992). The expression of Exp-1 in infected hepatocytes raises important questions regarding whether peptides from this protein expressed with HLA molecules on the surface of infected hepatocytes are the targets of protective T cell responses. T cells against the *Plasmodium yoelii* circumsporozoite protein can kill *P. yoelii*-infected hepatocytes (Weiss *et al.* 1990), and work is now underway to determine whether T cells that recognize peptides in Exp-1 can kill infected hepatocytes.

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